ON THE CANCER-PRODUCING FACTOR IN TAR.

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THAT tar can produce cancer has been known for nearly fifty years, but no systematic attempt to identify the particular compounds responsible for this effect was possible until the experimental production of cancer in lower animals became a practical method. In the absence of such experimental evidence from the laboratory, the capacity of any material to produce cancer must be learned from those accidental experiments on man which are the cause of "industrial diseases." When a new cancer-producing substance comes into industrial use on a large scale no danger will be detected during the long latent period, which in man is probably of many years' duration; the effect of the substance will then become apparent, as we have seen recently in the case of mule-spinners. The object of this paper is to bring together the evidence, from both the industrial and experimental sources, which indicates the presence or absence of carcinogenic power in the different fractions obtained from coal-tar and in the pure substances isolated from it.

SECTION I.—THE DIFFERENT FORMS OF TAR.

The forms of tar which are of most importance in the study of cancer are: (1) lignite tar, (2) gasworks tar, (3) producer-gas tar, (4) coke-oven tar, (5) blast-furnace tar. Of these, (1), (2), some forms of (3), and in all probability (4) produce cancer, while (5) does not. What, then, is the factor which determines whether a tar shall or shall not have cancer-producing properties? The composition and mode of formation of the various tars, and the occurrence of cancer in the workers handling them, cannot be dealt with in the present paper; but the lack of cancer-producing power in blast-furnace tar provides such important evidence that it is necessary to state the chemical characteristics of this tar.

Blast-Furnace Tar.

Blast-furnace tar does not produce cancer either in men (Legge[1]) or in mice (Leitch[2]). Unfortunately the composition of this tar has been little studied; almost all the current statements about it can be traced to the analyses of Watson Smith[3] published about forty years ago.

The chief differences between blast-furnace tar and the ordinary cancer-producing gasworks tar are as follows:

(*a*) The total quantity of phenols in blast-furnace tar is more than twice that in gasworks tar. Blast-furnace tar contains much less carbolic acid, but much more of the phenols other than carbolic acid (that is, cresols, xylenols, pseudo-cumenol, and alpha and beta naphthol). Thus the phenols other than carbolic acid make up over 98 per cent. of the mixed phenols of the carbolic-oil fraction of blast-furnace tar, and 35 per cent. of the corresponding portion of gasworks tar.

(*b*) Paraffins, liquid and solid, are present in much larger amount in blast-furnace tar. The solid paraffins make up about 1 per cent. of the tar, whereas they are absent, or present in traces only, in gasworks tar. The presence of paraffins is very undesirable if a tar is to be used as a source of benzene, toluol, or anthracene, and hence the amount of paraffin in gasworks tar is purposely kept as low as possible by maintaining a suitably high temperature during carbonization; in a blast furnace the temperature is, of course, not regulated by any such considerations.

(*c*) Of the aromatic compounds other than phenols: toluene, xylene, trimethyl benzene, aniline, and a small amount of naphthalene are present in blast-furnace tar, but Watson Smith could not detect either benzene or anthracene, which are, of course, very important commercial constituents of gasworks tar.

Thus a comparison of the composition of a cancer-producing and a non-cancer-producing tar shows that the latter contains the greater quantities of paraffins and of phenols other than carbolic acid, but is deficient in some aromatic compounds.

SECTION II.—THE FRACTIONS OF GASWORKS TAR.

The seven fractions which are produced by the ordinary commercial distillation of coal-tar are stated in Table I, where an attempt is made to indicate the relative amounts of the different fractions, and the temperatures between which each fraction is produced; the figures are only very rough, for the practice varies considerably in different tar-works, and also in the same works in accordance with fluctuations in the market for different products. Thus the proportion of creosote oil produced may vary from 9 per cent. to 25 per cent. of the crude tar.

TABLE I.—*Fractions of Gasworks Tar.*

Fraction.	Per Cent. of Tar.	Upper Limit of Temperature in Still.
1. Ammoniacal liquor	2	180° C. (1 and 2)
2. Crude naphtha	2	
3. Light oil	9	225°
4. Middle or carbolic oil	5	255°
5. Creosote oil	14	275°
6. Anthracene oil	3	320°*
7. Pitch	65	

* Steam distillation.

The *anthracene oil* is the only one of these fractions of which the further treatment by the tar-distiller must be described here. Anthracene oil contains a quantity of crystalline material in suspension. These solids, which amount to about 5 per cent. by weight of the oil, are separated from the oil by centrifuging or filtration, and constitute "40 per cent. anthracene." The oil is now known as green, strained, or dead oil; this is used for the manufacture of "grease" (see below) and for other purposes. The green oil is a saturated solution of the crystalline substances removed. The 40 per cent. anthracene is washed with solvents in which anthracene is not very soluble, and by this means is converted into "85 per cent. anthracene"; the material removed in this way, which consists chiefly of carbazole and phenanthrene, with acridine and of course many other substances, will be spoken of here as "anthracene residue."

The relationships of the derivatives of tar which must be dealt with in this section may be represented by the following scheme (Table II); those which are known to produce cancer are indicated by italics.

TABLE II.

Tar
- Crude naphtha.
- Light oil.
- Carbolic oil.
- *Creosote oil.*
- *Anthracene oil.*
  - *40 % Anthracene.*
    - 85 % Anthracene.
    - Anthracene residue.
  - *Green oil.*
- *Pitch* ... ...
  - *Pitch distillate.*
  - Pitch coke.

Distribution of the Cancer-producing Substance among the Different Fractions of Gasworks Tar.

The question then arises: Into which of these fractions does the cancer-producing substance present in the original tar pass? Cancer similar to tar cancer has been recorded in workers handling creosote, green oil, 40 per cent. anthracene, and pitch, and experimentally has been produced also by a high-boiling distillate from pitch which is not prepared commercially. We may now consider in detail the industrial and experimental evidence in the case of each fraction; when no other source is stated the experimental evidence is derived from investigations carried out in this institute.

1. *The Fractions of Low Boiling Point (Naphtha, Light Oil, Carbolic Oil).*

Industrial evidence appears to be almost wholly negative. Many of the substances contained in these fractions are present also in blast-furnace tar (toluene, xylene, trimethyl benzenes, cresols, xylenols). Ball[4] in 1885 reported that workers in a carbolic acid factory showed numerous warts; "one man, whose duty it was to fill the bottles with pure carbolic acid, had his hands covered with them." It is not clear whether these people came into contact also with the crude carbolic oil fraction or other tar products; no cases of cancer among them were reported. Story,[5] in the same year, described the case of a workman who received a splash of "crude carbolic acid" on the eyelid; at the spot a wart of the type familiar to carbolic acid workers developed, which in the course of a few months became an epithelioma; this was excised and examined microscopically. This seems to be the only case of cancer recorded in the literature which could be attributed to the fractions of coal-tar preceding the creosote oil. Possibly the cancer in this instance was of the type which occurs sometimes in scars, and was independent of the chemical nature of the injury.

Experimental evidence with regard to this and the next fraction (creosote) may be taken together. Bloch and Dreifuss[6] refer to experiments in which mice were painted with "the fraction of low-boiling hydrocarbons"; they do not state any limits of temperature, but it is evident that some fraction boiling below 300° is meant. Whether this indefinite term is intended to include the creosote must be left uncertain; this fraction of tar would include many substances which are not hydrocarbons at all. This fraction produced tumours which ulcerated late and were as a rule non-malignant in character; there was a considerable development of the stroma and blood vessels, so that such tumours might be given the name "strongly vascularized benign fibro-epithelioma."

In this institute we have obtained negative results with xylol (boiling point about 136° C.); and we can exclude the constituents of "90 per cent. benzol" (that is, benzene, toluene, xylene) which has been used as the solvent for several substances (for example, acridine, phenanthrene) which have given negative results in tests of nine months' duration. Apparently no conclusive experiments have been made with the creosote fraction alone.

2. *Creosote Oil.*

Industrial Evidence.—MacKenzie[7] recorded the case of a man engaged in treating timber with creosote, on whose scrotum were several papillomata "from the size of a pea to that of a nut." Apparently this author had met with other such cases in creosote workers, in one of whom a similar tumour became an epithelioma, and was excised; unfortunately, this second case is only vaguely described, and it is not quite clear whether the man worked with creosote or with tar. Legge[8] recorded cases of eczematous eruptions of the forearms in men using creosote to lubricate die-moulds in a brickworks; in one old man an epithelioma developed on the forearm, and was excised. In the following year a case of epithelioma, and another of severe ulceration of the forearms and hands, were reported to the Home Office from a "creosoting factory."[9] O'Donovan[10] described cancer in three men employed in creosoting timber; the occupation of a fourth is described only by the words "pickles telegraph poles," no substance being named, but in all probability this was creosote. In three of these men the cancer was on the scrotum, while the fourth man had one cancer on the lip and three on the arms.

[Since this section was written a good account of a case of creosote cancer, with illustrations, has appeared in this JOURNAL (Cookson, BRITISH MEDICAL JOURNAL, March 1st, 1924).]

3. *Anthracene Oil and Green Oil.*

Industrial Evidence.—Green oil, and sometimes creosote also, is mixed with rosin and lime to make lubricating greases; heat is evolved when the lime is added to the oil, and the arms of the workmen are often splashed by the hot liquid. Oliver[11] described two cases of epithelioma of the arm among grease-makers, of which one ended fatally. Unfortunately the material used is described only as "oil distilled from coal," but in all probability it was green oil or creosote or both of these. Legge,[12] in describing an examination of the workmen in eight oil and grease works where "coal or anthracene oil" was used, says: "One man using grease in the filling of brattice cloth was found with extensive ulceration of the arm and a scar marking the place where an epithelioma had been removed." These records are sufficient to show that the practice, common in some tar works, of removing tar from the skin with heavy oil,[13] is not free from danger unless the oil be afterwards removed completely.

Experimental Evidence.—Both anthracene oil and green oil were found to be very toxic to mice; in each case three mice only out of 100 lived more than six months. One epithelioma appeared in each series. Thus both oils are capable of producing cancer, but the number of mice surviving in the later months was too small to allow any conclusion to be drawn as to the relative potency of the two.

4. *40 per cent. Anthracene and Anthracene Residue.*

Industrial Evidence.—The only cases recorded in this country of epithelioma due to crude anthracene seem to be those described by O'Donovan.[14] Three men engaged in handling 40 per cent. anthracene in an alizarin factory developed epithelioma on the hand, cheek, and wrist respectively; one of the three had previously been a pitch-breaker; of the two others, one had worked for thirty years and the other for thirty-two years with the crude anthracene, and these two had never worked with any other tar product. Of the twenty-five men employed in the factory, those dealing with the purified anthracene were free from the skin lesions (acne, keratoses, telangiectases, pigmentation) which affected those in contact with the crude material. Leymann[15] mentions three workers in the anthracene department of a German chemical factory who had to undergo operation for tumour of the scrotum, but he gives no further information.

Experimental Evidence.—*40 per cent. anthracene* was tested upon two series of 100 mice; one series received a suspension in lanolin and showed no tumours; the other received an ethereal extract, and after five months one of these mice showed a papilloma bearing a long horn; no other tumours appeared. An ethereal extract of *anthracene residue* produced no tumours; this test was not very satisfactory, as only 8 mice out of 100 lived longer than 160 days. But these experiments are sufficient to show that the cancer-producing substance is not concentrated among the solids in suspension in anthracene oil.

5. *Pitch.*

Industrial Evidence.—Of the 95 cases of epithelioma notified to the Home Office from England and Wales between January, 1920, and June, 1922, 66 were in pitch workers (Legge[16]). Many writers have described the conditions under which the pitch-breakers and the briquette-makers work, and the affections of the skin which they show (Legge,[12] Warnes,[17] Ehrmann,[18] and others), so that it is unnecessary to give any account of the matter here. Probably pitch cancer could be almost wholly eliminated if one of the systems now in use in some works in Holland and Germany[15 19] could be adopted. Thus in the Fohr-Kleinschmidt system the liquid pitch can be run off from the tar-stills into tank wagons and conveyed to a briquetting plant, where it is converted in a closed apparatus, which is patented, into a fine dry powder; this is mixed with the coal-dust in a closed drum. Thus the work of the pitch-breaker at the gasworks is abolished altogether, and the briquette-makers are not exposed to pitch-dust.

Experimental Evidence.—Pitch was applied to two series of 100 mice. Series A received a suspension of pitch in melted lanolin (3 grams in 10 c.cm.). Between the 181st day, when 27 mice were alive, and the 294th day 9 tumours appeared; of these, 3 were papillomata, of which 2 showed considerable downgrowth, while the remaining 6 were typical epitheliomata. Dr. Leitch has found that a suspension of pitch in the sebaceous secretion obtained from dermoid tumours is equally effectual in producing cancer. Series B received the filtered solution from pitch (3 grams) in "90 per cent. benzol" (10 c.cm.); this produced papillomata very quickly, the first appearing on the sixty-fourth day. This preparation was far more toxic than the other; only two mice lived more than 150 days. Two tumours were examined, one being a papilloma and one an early carcinoma. Jordan[20] also has produced cancers by means of a solution of pitch in benzol.

6. *Pitch Distillate.*

Experimental Evidence.—When pitch is heated in an iron retort the first distillate which comes over is greenish-black and greasy, like anthracene oil. If the temperature be raised to 490° C. (bulb of thermometer in liquid) the distillate becomes yellowish, and at 510° C. a clear orange-yellow liquid, having no resemblance to anthracene oil, comes over; this solidifies to a reddish material rather like sealing-wax. This liquid continues to distil over till 550° C. is reached; the residue in the retort is then a very hard coke. A solution of the red solid in 90 per cent. benzol, in which it is almost wholly soluble, was used for application to 100 mice; between the fifth month, when 12 mice remained alive, and the twelfth month, when the last was killed, 4 epitheliomas and 2 papillomas with considerable downgrowth were obtained.

Summary of Section II.

The industrial evidence alone shows that the cancer-producing substance is present in the higher boiling fractions—namely, creosote oil, anthracene oil (and hence in the green oil and crude anthracene), and pitch; the experimental evidence supplements this by showing that the substance is not concentrated in the solids suspended in anthracene oil, and that it is present in the highest boiling distillate obtainable from pitch. Hence it may distil over through an interval of temperature extending roughly from 250° C. (the creosote fraction) to above 500° C. (the "pitch distillate")—that is, over a range of 250° or more.

SECTION III.—A COMPARISON OF THE COMPOUNDS OF HIGH BOILING POINT IN TARS AND MINERAL OILS.

Since cancer is produced by gasworks tar, lignite tar, shale oil, and at any rate some forms of petroleum, it is natural to inquire (1) what substances are common to all these liquids; and (2), since blast-furnace tar is innocuous, and the evidence points to the comparative feebleness of crude petroleum in this respect, what substances are absent or less abundant in these two. With this object in view a list as complete as possible was compiled of the compounds stated in the literature to be present in each of these five liquids. This piece of work obviously had to be carried out, but the result was not very rewarding.

The chief difficulties were as follows: (1) because any compound has not been demonstrated in, say, shale oil, one can by no means assume that it is not present; (2) we have very little information about the composition of blast-furnace tar and of shale oil; (3) many of the statements about lignite tar refer to the modern product, and it is not certain whether this produces cancer; the most recent case of lignite-tar cancer of which any record has been found in the literature occurred thirty-four years ago; (4) the whole subject of petroleum is made difficult by the great number of different kinds of oil, differing very widely in composition, and by the scantiness of the information available about many of them.

Attention was then directed especially to the compounds of higher boiling point, for it seems clear from the facts given in Section II of this paper that the cancer-producing substance must be sought amongst these. As an arbitrary lower limit, a boiling point of 270° C. (roughly the temperature at which the distillation of anthracene oil is begun) was chosen, and a separate list made of all the compounds known to be present in gasworks tar which boil at or above this point.

These compounds are the following: fluorene, fluoranthene, acenaphthene, anthracene, β-methyl anthracene, anthracene di- and hexa-hydride, phenanthrene, retene, pyrene, chrysene, picene, truxene, naphthacene, naphthanthracene, chrysogene, crackene, benzerythrene, α- and β-naphthol, β-naphthofuran, diphenylene oxide, carbazole, phenyl-naphthyl-carbazole, acridine, a dimethyl quinoline, diphenylene sulphide, and some of the higher paraffins in very small amounts. Apparently no members of the olefine, acetylene, or naphthene series having boiling points above 270° C. are known to occur in coal-tar.

We must now consider which of the compounds in this list are common to the other cancer-producing fluids and to blast-furnace tar. The only substances boiling above 270° which are stated to be present (*a*) in blast-furnace tar are the naphthols; (*b*) in lignite tar are picene and chrysene; (*c*) in Scottish shale oil are pyrene and chrysene (the literature does not seem to contain adequate proof of the identification of these compounds in shale oil), and the methyl quinolines from $C_{11}H_{11}N$ onwards; with some higher paraffins in each case.

In some of the petroleums, compounds boiling above 270° of the paraffin, olefine, and terpene series are present; but apparently no substance on the above list has as yet been shown beyond doubt to be present in any *unheated* oil. In the distillation residues, on the other hand, such as those obtained by distilling Pennsylvanian oil in red-hot iron retorts, anthracene, phenanthrene, chrysene, and pyrene are present, and the same substances have been found in the Galician residues (Höfer[21]). The cases of petroleum cancer recorded by Ullmann[22] and by Derville and Guermonprez[23] occurred in men engaged in removing such residues from the stills. The cancer-producing power of a large number of petroleums is being tested by Dr. Leitch in this institute.

This comparison of the composition of gasworks tar, lignite tar, and Scottish shale oil shows, then, that chrysene, picene, and pyrene are common to two or more of them. The results of experiments in this institute with these and other high-boiling substances have so far been wholly negative. Each substance is tested upon a series of 100 mice, and the experiment is continued for nine months.

Chrysene (a 1 per cent. solution in warm "90 per cent. benzol" of a preparation by Kahlbaum) is distinctly toxic; 10 mice were alive after three months, and 2 mice after eleven months, when the experiment was stopped; none showed any signs of a tumour. *Picene* and *retene*: Dr. Chattaway of Queen's College, Oxford, has been so kind as to give his very pure preparations of these two hydrocarbons to be tested; at the present date, after six months' application of solutions in sebaceous material from dermoids, the results are quite negative. *Pyrene* has not yet been tested here; Bloch[24] records a negative result with this compound. *Anthracene* dissolved in olive oil gave a negative result; this is in agreement both with the fact that workmen handling purified anthracene do not suffer from skin lesions (see above under "40 per cent. anthracene") and with the experiments of Fibiger,[25] who failed to produce tumours in mice and rabbits by subcutaneous injections of suspensions or solutions of anthracene in oil.

Truxene seemed to be of considerable interest, for it, or compounds allied to it, have been thought to be important constituents of pitch. It was synthesized from phenyl-propionic acid and applied in solution and suspension in various media (lanolin, xylol, 90 per cent. benzol) without result.

Phenanthrene, fluorene, and *acenaphthene*, dissolved in 90 per cent. benzol, all gave negative results; the two latter are distinctly toxic. *Acridine* has often been suggested as the cancer-producing agent in tar, probably on account of its irritating effect upon the skin and mucous membranes. It was applied, dissolved in lanolin in the one case and in 90 per cent. benzol in the other, to two series of mice—that is, 200 in all; the only noticeable effect was that the health of these mice was especially good, so that an unusually large proportion lived throughout the nine months. This provides another illustration of the fact that "irritation" alone is not a means of producing cancer (cf. the chrome workers—Legge[16]).

The formula of *carbazole* suggested the possibility of establishing a connexion between the forms of cancer affecting aniline workers and tar workers, but no effects upon the skin or bladder were produced by a 1 per cent. solution in alcohol; subsequently evidence indicating that the cancer-producing substance does not contain nitrogen was obtained (see below). Thus, of the high-boiling substances given in the list above, the following have already been tested in this institute with negative results: fluorene, acenaphthene, anthracene, phenanthrene, retene, chrysene, picene, truxene, carbazole, acridine. The remaining seventeen substances in the list have not as yet been available for experiment; of them one can exclude the naphthols, for these are present in blast-furnace tar.

In conclusion, a few points about some other compounds and classes of compounds may be considered. *Naphthenes* are present in very small amount in gasworks tar. They are present in specially large amounts (up to 80 per cent.) in Russian (Baku) petroleum; none of the very few cases of petroleum cancer of which we have been able to find records were due to Russian petroleum. The *olefines* are present in very small amount in high-temperature tar, but more abundantly in Scottish shale oil; the only petroleum at all rich in them is the Californian, which certainly has not attracted notice as a source of industrial cancer, and has given negative results in Dr. Leitch's experiments on mice in this institute.

With regard to the *paraffins*, it may be noted that high-temperature coal-tar, which, together with its pitch, produces far more cases of industrial cancer than do all other materials put together, is especially poor in paraffins and contains less of them than does blast-furnace tar, which does not produce cancer. The evidence on this point derived from the lignite tar and Scottish shale oil industries will be given more fully in another paper; in both these industries the workmen who have most to do with the most concentrated forms of paraffin are the least liable to affections of the skin. The petroleums of Pennsylvania, Canada, Galicia, and Rumania contain especially large amounts (up to 85 per cent.) of paraffins, but are not distinguished by the production of many cases of petroleum cancer; we have been able to learn of one case from the United States, and of 13 cases in ten years in twenty refineries in a district of Galicia (Boryslaw-Tustanowice), where the oil contains about 32 per cent. paraffin (Ullmann[22]), which is about three times the amount present in Scottish shale oil. Prosser White[26] (p. 143) says: "The writer has watched women and girls employed all their working hours, year after year, bedaubed with refined paraffin wax free from impurities, none of whom have experienced the least trace of inconvenience or distress." Deelmann[27] found that horizontal-retort tar from the Amsterdam gasworks produced malignant tumours in mice much more rapidly than did the vertical-retort tar; average samples of the former variety of tar contain a slight trace of paraffins only, and of the latter about 0.3 per cent. (West[28]). Weidmann and Jefferies[29] have given a valuable summary of the results of subcutaneous injection of paraffins in man and in monkeys; the type of lesion observed so far "is a foreign body granuloma, and in no sense of the word neoplastic." It is unfortunate that the idea that paraffins cause cancer should be encouraged by the use of the term "wax cancer" in the title of a paper ("Paraffinoma and Wax Cancer" by Davis[30]) which does not contain a description of even a single case of any condition which could be described as "wax cancer." The degree of accuracy which is considered sufficient in reporting cases of occupational cancer is shown very well by a passage in the thesis by Scott,[31] who examined the records of nearly a quarter of a million hospital admissions: "In examining the records of Edinburgh Royal Infirmary for the past twenty-two years, numerous cases have been recorded as paraffin cancer among railway workers, dock labourers, masons, chimney-sweeps, glass makers, enginemen, etc., in none of whom was the condition likely to have been due to paraffin."

Naphthalene seems to be excluded by its great volatility, which causes it to be present in the lower-boiling fractions; by its presence in blast-furnace tar; and by the fact that the men engaged in its manufacture show only negligible affections of the skin (Prosser White,[26] p. 150). Bierich[32] produced cancer in mice once with *pyrrole* and once with *benzidine*; he tested twenty-seven other substances with negative results, but unfortunately he does not name them. Our results with benzidine and *aniline* were wholly negative, both as regards the skin and the bladder; benzidine has not been found in coal-tar, and aniline is present in blast-furnace tar.

Later experiments have shown that at any rate a very large part of the acids and bases can be removed from tar without destroying its cancer-producing power. Tar was extracted by shaking, for six hours in each case, first with water, then with alcohol, and finally with ether; such ether extracts are very active in producing cancer (Murray[33]). The ether extract was distilled fractionally, and certain fractions—namely, fraction C, obtained between 350° and 400° C., and D, between 400° and 450° C.—chosen for further treatment. These fractions were shaken out by hand (1) four times with 15 per cent. NaOH; (2) twice with water; (3) four times with 20 per cent. H_2SO_4 by weight; (4) twice with water. C and D after this extraction of acids and bases were dissolved in ether and applied to series of 100 mice. In the "D" series, between the 120th day when 30 mice were alive and the 258th day when 5 mice remained, 8 mice developed epithelioma and one a non-malignant epithelial horn; this is as large a yield of tumours as one would obtain with many samples of crude tar. Fraction C at the present date (355th day) has produced one epithelioma, presenting some remarkable features which will be referred to in a later publication, and among the 9 surviving mice there is one tumour which is almost certainly malignant, and one papilloma. Thus the higher-boiling fraction D is the more active. The ordinary Prussian blue test shows a very slight trace of nitrogen in C, and a distinctly larger amount in D. Bloch[24] mentions cancer-producing fractions of tar containing no acids, bases, nor nitrogen, and a trace only of sulphur, but he gives no details.

Summary of Section III.

Thus the attempts made as yet to find the cancer-producing substance among the known constituents of coal-tar have given wholly negative results. Anthracene, phenanthrene, chrysene, picene, retene, truxene, acenaphthene, fluorene, acridine, carbazole, aniline, benzene, toluene, and xylene have been excluded by experimental tests; and either the experimental or industrial evidence, or both of these, is against the importance of naphthalene, the acids, bases, and other nitrogenous compounds, paraffins, olefines, and naphthenes. It now seems not unlikely that this substance is a compound, as yet unknown, which is unstable and present in amounts perhaps as small as those of the vitamins in foods; as in the case of some hormones, its identification may be long delayed even when very concentrated preparations can be obtained. The substances which are known to be present in coal-tar have been isolated because they are specially abundant, or stable, or capable of forming well defined compounds; the unknown substances are unknown because they have not these properties, and the cancer-producing substance may well be among them.

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THE RELATION OF MANURE TO THE NUTRITIVE AND VITAMIN VALUE OF CERTAIN GRAIN.

BY

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THE distribution of true tropical beri-beri in India[1] having led me to suspect that the nutritive and vitamin values of food grains might possibly vary with the conditions of their growth, an attempt was made, during 1923, to ascertain whether or not these values varied with the manures used in their cultivation. Unfortunately the research had to be stopped, in consequence of recent financial retrenchments in India, before it could be completed; nevertheless, the results were so consistent that I venture to record them, without, however, desiring, in the absence of further work, to assert that they are to be regarded as final.

At the suggestion of Dr. R. V. Norris, agricultural chemist to the Madras Government, the permanent manurial plots at the Central Farm, Agricultural College, Coimbatore, Madras, were used for the purpose. To him I am indebted for the samples of millet (*Eleusine coracana*) grown on these plots and used in the experiments to be described. He has also provided me with the following details relating to soil, manure, cultivation, and yields of the respective plots, and with analyses of the millet grown upon them.

Soil and Manure.

There are ten permanent manurial plots, each four cents in area.[2] They were laid down in 1909,[2] and had been under observation for fourteen years at the time the present investigation was commenced. During this period thirty-nine crops had been raised. The soil is a fairly heavy loam, typical of much of the land in the Coimbatore district, and characterized by a low content of organic matter and a deficiency of nitrogen and available phosphoric acid. Eight of the plots received mineral manures—that is, nitrogen, phosphoric acid, or potash, either alone or in combination—while the last two have been treated with cattle manure. The application of cattle manure to plot x, however, was stopped in 1916, "in order that the residual effect of the manure might be studied." The treatment of the ten plots was therefore as follows:

Plot I: No manure.
,, II: Nitrogen.
,, III: Nitrogen *plus* potash.
,, IV: Nitrogen *plus* phosphate.
,, V: Nitrogen *plus* potash *plus* phosphate (complete chemical manure).
,, VI: Potash *plus* phosphate.
,, VII: Potash.
,, VIII: Phosphate.
,, IX: Cattle manure (continuous).
,, X: Cattle manure (discontinued since 1916).

The manures were used in the following proportions: (1) Ammonium sulphate: 1 cwt. per acre, supplying 20 lb. of nitrogen. (2) Superphosphate: 3 cwt. per acre, supplying 50 lb. of phosphoric acid. (3) Potassium sulphate: 1 cwt. per acre, supplying 50 lb. of potash. (4) Cattle manure: 5 tons per acre, supplying approximately 70 lb. of nitrogen, 40 lb. of phosphoric acid, and 100 lb. of potash. The cattle manure was a farmyard manure—that is, a mixture of dung, urine, and litter. The manures were applied at the end of the preliminary cultivation one day before the crop was transplanted.

Cultivation.

The plots are intensively cultivated by hand, three crops being taken off each year. Irrigation is supplied as required. In the particular crop of millet under consideration preliminary cultivation took place in July, 1922; the manures were applied on August 7th and the millet seedlings were transplanted on August 8th. Irrigation was given on seven occasions, and the crop was harvested on November 12th, 1922.